THE RELATION OF THE AMINO ACIDS TO NITROGEN EQUILIBRIUM

BY

GLENN FREDERICK LAMBERT

A. B., De Pauw University, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
OF ILLINOIS, 1944

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PART I

THE MAINTENANCE OF NITROGEN EQUILIBRIUM IN DOGS BY INTRAVENOUS ALIMENTATION WITH MIXTURES OF AMINO ACIDS

Introduction

In certain disorders of the gastro-intestinal tract it is impossible to give the patient any food by mouth. To prevent a serious breakdown of the tissues, nitrogen must be administered by some other route. Intravenous alimentation with a mixture of the indispensable amino acids should fulfill this requirement. It has been reported that the intravenous administration of a casein hydrolysate or a mixture of crystalline amino acids to men or dogs leads to the regeneration of plasma proteins[1], and the production of positive nitrogen balance[2,3,4]. The data reported in the literature are inconclusive because the experiments did not occupy a sufficient length of time and the exact composition of the casein hydrolysate was not determined. The present study was undertaken to investigate this problem with a carefully controlled experiment using mixtures of purified amino acids.

Experimental

Adult female dogs were used throughout. Nitrogen balance and the partition of the urinary nitrogenous compounds were studied. Complete twenty-four hour urine samples were collected by catheterization while the feces were divided into periods by the use of carmine capsules.

A mixture of either nine or nineteen amino acids supplied the nitrogen of the diet. The mixture under consideration was fed orally for a seven-day period and was then given by vein to the same animal for the following seven days. In this man-

ner a comparison of intravenous and oral feeding was obtained. In all cases the remaining food constituents were given by mouth. The injection solutions of amino acids were approximately isotonic. Nitrogen intake was maintained constant at 150 mgm. per kilo of body weight.

Two commercial casein hydrolysates were also investigated. It was necessary to determine the minimum quantity of each that would maintain positive nitrogen balance when given orally. This amount was then administered intravenously.

Summary

It has been shown that nitrogen balance can be accomplished and maintained in dogs by intravenous alimentation. A mixture of the nine essential amino acids has proved as effective as a more complex mixture of nineteen protein components. However, a slow rate of injection must be used to attain positive balance and prevent the production of nausea. It is best to administer the solution by vein in two equal portions separated by an interval of four hours. Only a very slight increase in amino acid excretion occurs when intravenous alimentation replaces oral administration.

The two commercial hydrolysates of proteins studied have not been found to be so efficient in the maintenance of nitrogen balance when given by the intravenous route as are appropriate mixtures of purified amino acids. One product was deficient in tryptophane. In the case of the other, a considerable excess over the minimum oral requirement was found necessary when the mixture was administered intravenously. Furthermore, a large increase in amino acid excretion occurred when intravenous alimentation replaced oral administration.

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PART II

THE INABILITY OF α-CHLOROISOCAPROIC ACID AND ISOPROPYLACRYLIC ACID TO REPLACE LEUCINE OF THE DIET FOR GROWTH PURPOSES

Introduction

It is possible to use the method of growth as an index of the effectiveness with which a compound may be substituted for an indispensable amino acid in the diet of the rat. Thus, two α,β-unsaturated acids have been tested in this manner. Rose and Cox[1] found that β-4-imidazoleacrylic acid cannot replace histidine while Berg[2] has reported thatβ-3-indoleacrylic acid cannot be substituted for tryptophane. No α-chloro acids have been investigated by this method.

In the present study, the ability of α-chloroisocaproic acid and isoproplacylic acid to replace leucine was determined.

Experimental

Albino rats 26 to 29 days old, weighing 43 to 61 gm. each, received diets which were devoid of protein but contained a mixture of highly purified amino acids. The leucine of the ration was replaced by α-chloroisocaproic acid or sodium isopropylacrylate. The growth rate of the animals was compared to that of positive controls receiving leucine and negative controls receiving no leucine. Feeding experiments were conducted for a period of 28 days.

Summary

Neither α-chloroisocaproic acid nor isopropylacrylic acid can replace leucine in the diet of the rat for growth purposes. The α-chloro acid is extremely toxic, producing death within a few days.

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PART III

THE QUANTITATIVE TRYPTOPHANE REQUIREMENT OF THE ADULT HUMAN

Introduction

Previous studies have shown that only eight of the amino acids occurring in proteins must be present in the diet of adult men for the maintenance of positive nitrogen balance[1,2,3]. The present investigation is a study of the quantitative tryptophane requirement of human subjects.

Experimental

Two adult males served as subjects. The diets contained over ninety-five per cent of the nitrogen in the form of mixtures of purified amino acids, and consisted of amino acids, lemon juice, butter, corn starch, sucrose, corn oil, minerals, cellu flour, cod liver oil, and a mixture of vitamins. The caloric intake remained constant throughout the experiment at a level of 55 calories per kilogram of body weight. Distilled water was taken *ad libitum*.

Nitrogen balance studies were performed. Twenty-four hour urine samples were collected while the feces were separated into periods by the use of charcoal tablets.

The amino acid mixtures were composed of the eight indispensable amino acids in addition to histidine and glycine. The tryptophane level was gradually decreased until a negative nitrogen balance was obtained, and was then raised until positive balance again resulted. The glycine content was varied to compensate for these changes, and thus the total nitrogen intake per day remained constant at 9.99 gm.

The ability of man to utilize *d*-tryptophane was studied by replacing the minimum requirement of *l*-tryptophane by an equal quantity of *dl*-tryptophane.

Summary

Both subjects exhibited negative nitrogen balance at a level

of 0.10 gm. of *l*-tryptophane per day, while 0.15 gm. produced positive balance. A daily intake of 0.20 gm. of *l*-tryptophane is recommended.

The *d*-isomer of tryptophane cannot be utilized by the human.

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Vita

The author was born November 21, 1918, in Columbus, Ohio. He received his elementary and secondary education in the public schools of Fort Wayne, Indiana. He entered DePauw University as a Rector Scholar in September, 1936, and received the Bachelor of Arts Degree in June, 1940. He entered the graduate school of the University of Illinois in September of 1940 as a Special Research Assistant in Chemistry. He held this appointment for the succeeding three years. During the summer semester of 1943 and the first semester of 1943-1944 he was a University of Illinois Research Fellow in Chemistry.